Reference: *Biol. Bull.*, **136**: 253–263. (April, 1969)

SENSE ORGANS ON THE ANTENNA OF A PARASITIC WASP, *NASONIA VITRIPENNIS* (HYMENOPTERA, PTEROMALIDAE)[1]

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One of the objectives of the series of studies on the sense organs of insect antennae undertaken by the writer is to discover whether insects occur that lack chemoreceptors of a particular type or, conversely, whether any kind is universally present. Thin-walled chemoreceptors with many fine pores in their cuticle, where filaments from the olfactory dendrites end, have been found in every species so far examined intensively (Slifer, 1967 review; 1968a, 1968b; Myers, 1968). Their cuticular parts take the form of hairs, pegs or plates that are domed, raised or flattened. Similarly, thick-walled chemoreceptors have been identified in each of the species listed in the references given above. These consist of hairs, pegs or bristles and have a single pore at the tip where the dendrites are exposed to the air. Some arise from the surface of the antenna while others, usually pegs, are set in cavities where the antennal cuticle is invaginated so that the tip of the peg lies below and sometimes at a considerable distance from the surface of the antenna.

A preliminary examination of the antennae of *Nasonia vitripennis* suggested that this was a species that lacks thick-walled chemoreceptors although thin-walled chemoreceptors are present in abundance. It was not until the study was nearing completion that a few thick-walled chemoreceptors were found.

Materials and Methods

Samples of adult *Nasonia vitripennis* (Walker) from wild type and scarlet stocks were kindly given the author by Dr. Anna R. Whiting who is now at the Oak Ridge National Laboratory in Tennessee. [*Nasonia vitripennis* (Walker) is also known as *Mormoniella vitripennis* (Walker) and the latter name is frequently used in current literature.] Some of these were fixed in Bouin's solution and others in 5% formalin.

Some antennae were stained in borax carmine or with the Feulgen technique and mounted whole. Others were examined in glycerol which usually makes pores in the thin-walled receptors easier to see. Entire insects were treated with a 0.5% solution of crystal violet for the identification of pores in the cuticle of sense organs on the antennae and elsewhere (Slifer, 1960). Males of the scarlet stock used have pale antennae and it is easier to study whole mounts made with them than with dark wild type antennae. Antennal flagellae were embedded in Paraplast, sectioned at 5 or 7 μ and stained with Holmes' silver method, Mallory's stain or Heidenhain's iron-hematoxylin.

[1] Supported in part by a grant from the National Science Foundation GB-7310.

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Results and Discussion

Nasonia vitripennis is an insect of economic importance since its larvae destroy the pupae of Diptera by developing within them and of scientific interest because it has been much used in laboratories for studies in genetics and related fields. A valuable review, listing over 200 references to earlier work on the species, has been published by Whiting (1967).

The antenna of the female *Nasonia* was described by Jacobi (1939). In general, the present paper confirms his results but information and techniques that were not available thirty years ago now permit a more detailed description and better understanding of the function of the various sense organs. The male antenna is also included.

In both sexes the antenna consists of a long scape, a shorter pedicel and a flagellum composed of twelve subsegments (Fig. 1). The first and second flagellar

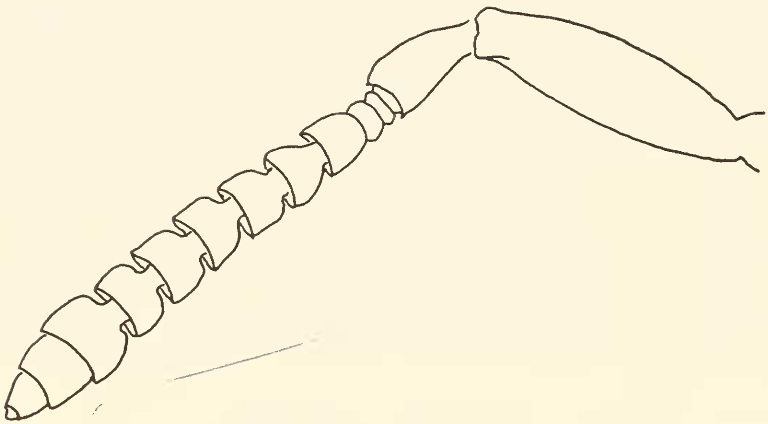

Figure 1. Right antenna of male as seen from medial surface. Scape (right), pedicel and flagellum with twelve subsegments. Sense organs omitted. × 168.

subsegments are short and, like the scape and pedicel, provided only with hairs believed to be tactile. Olfactory receptors, together with a few tactile hairs, are numerous on the remaining subsegments. Subsegments three to eight are each constricted sharply at their distal and proximal ends. Subsegments nine to twelve, in contrast, form a compact unit and lack deep constrictions between them although their boundaries are clear. The twelfth subsegment is very small (Figs. 1, 3) and, instead of being placed symmetrically at the apex of the antenna, the greater part of it faces medially and ventrally. Although *Nasonia* males are distinctly smaller than are the females the antennal flagellum is nearly the same length in both sexes —about 350 μ for the material examined here.

Five structurally different types of sense organs occur on the flagellum of both males and females (Fig. 2). Two of these are almost certainly tactile in function and the other three have the characteristics of chemoreceptors. Coeloconic and ampullaceous sense organs are commonly present on the antennae of Hymenoptera but none were found in *Nasonia* by Jacobi (1939) nor by the present writer.

Tactile hairs

Although these are very small and it is impossible to see with the light microscope that they possess all of the structural features of tactile organs proven to be such in other species, enough evidence can be obtained to assign this function with a high degree of certainty. They are of two kinds. The first (Figs. 2a, 3e) is slender, nearly straight, sharp-tipped, about 12 μ long and has a basal diameter close to 0.5 μ. It was described by Jacobi (1939) as a *kleines Haar* that is probably tactile. His drawing of a longitudinal section through the hair shows only its outline with an indistinct strand of material entering its base. When the intact insect is immersed in a solution of crystal violet the dye does not enter these hairs. They are present on each of the flagellar subsegments except the twelfth. The same type of hair, usually larger and stouter, occurs on the scape, pedicel and other parts of the body. Few satisfactory sections that included both one of these hairs

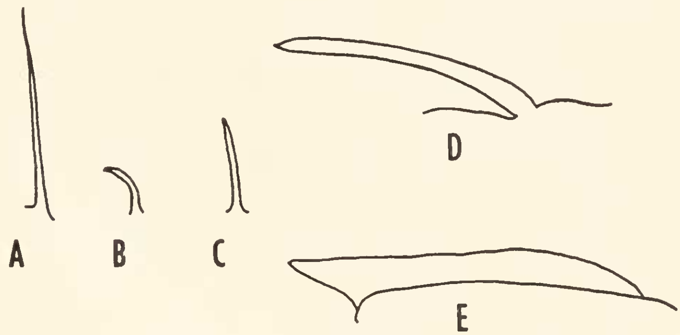

FIGURE 2. Five types of sense organs found on flagellum. All drawn in lateral view and with surface directed toward distal end of antenna at left. A, slender hair with sharp tip, probably tactile; B, curved hair, probably tactile, from twelfth subsegment; C, thick-walled chemoreceptor with opening at tip from twelfth subsegment; D, thin-walled chemoreceptor; E, plate organ. × 1452.

and the cells associated with it were obtained. Sometimes a nerve strand could be seen extending below the hair base and ending in a group of three or four cells. This strand is so exceedingly thin that it is improbable that it consists of more than one dendrite from a single sensory cell. A tactile receptor, as is well known, is usually innervated by one neuron. The other two or three cells are probably sheath cells.

Jacobi (1939, Figs. 28, 29) found two kinds of hairs, both small and thin-walled, on the twelfth subsegment. He suggests that both are tactile. The smaller of the two probably does have that function. It is sharply curved, from 5 to 7 μ long and has a basal diameter of 0.5 μ (Figs. 2b, 3b). The tip is slender but not so sharp as that of the hair described in the preceding paragraph. The hairs do not stain when the insect is treated with crystal violet and this indicates that they lack pores where olfactory dendrites are exposed. The cellular parts of these receptors could not be distinguished from those of the other sense organs close to them. According to Jacobi (1939), the female beats or drums rapidly with the antennal tips on the dipteran pupal shell when hunting for a suitable place to lay eggs. These small curved hairs on the end of the twelfth subsegment would be ideally located and constructed to serve as tactile organs for distinguishing differences in the pupal covering. In the male, contact of the antenna with the female is important in courtship (Barrass, 1960) ; but whether the antennal tip is especially concerned is not known.

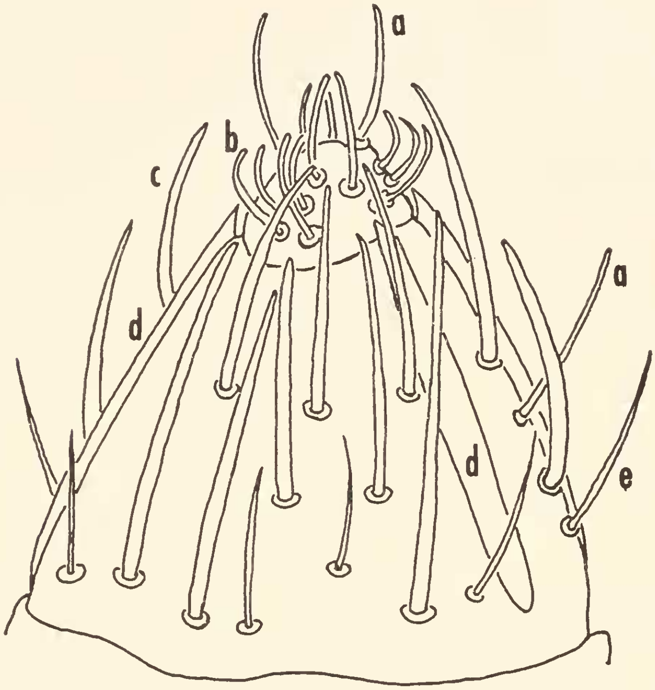

FIGURE 3. Eleventh and twelfth subsegments of male to show all types of sense organs. A, thick-walled chemoreceptor ; B, short, curved hair ; C, thin-walled chemoreceptor ; D, plate organ ; E, slender, sharp-tipped hair. Sense organs of subsegment ten that extend forward over the eleventh have been omitted. × 1605.

Chemoreceptors

Three types of chemoreceptors—thick-walled pegs, thin-walled pegs and plate organs with a thin wall—are present on the antennal flagellum of *Nasonia.* The first and second are smaller in size, number and distribution in both sexes while the third occurs in larger numbers in the female than in the male.

I. Thick-walled pegs

These are found only on the eleventh and twelfth subsegments (Figs. 2c, 3a) and are the larger of the two kinds listed by Jacobi (1939, Fig. 29) as present on the terminal subsegment. About six are located on the extreme tip of the flagellum and, since they are longer than the curved hairs described in the preceding section, would be the first to come into contact with any surface to which the end of the antenna is touched. Five or six others occur on the eleventh subsegment. They are about 9 μ long, 1 μ wide at the base, slightly curved and, in contrast to the tactile hairs, have a tip that is rounded. When a solution of crystal violet is applied to the external surface of the antenna, stain enters the tip of the thick-walled peg and, with continued exposure, passes downward towards the base. This indicates that a pore is present at the tip where the dendrites are exposed. The sensory cells innervating these receptors apparently lie within the large mass of neurons present in the tenth and eleventh subsegments and cannot be identified separately. In other species of insects, similar thick-walled chemoreceptors are usually provided with four, five or six neurons. Thick-walled pegs have been found in all species in which a search has been made for them. Those on the mouth-parts and tarsi have been studied intensively, especially in Diptera, by Dethier (1955) and by many others. They occur not only on the antenna but on many other parts of the body as well (Slifer, 1955, 1962). They serve both as olfactory organs and as contact chemoreceptors. Their concentration on the antennal tip in *Nasonia* should aid in the exploration of the surface of the dipteran puparium by the female and may assist the male in recognizing the female.

Since thick-walled pegs occur on many parts of the body besides the antennae in other species, intact *Nasonia* were treated with crystal violet to see whether the pegs were present on the mouth-parts and legs. A single thick-walled chemoreceptor was found on the upper surface of each of the tarsal claws, about five on the terminal portion of each maxillary palp and three or four on the tip of each labial palp. Jacobi (1939) suggested that olfactory organs are present on the palps since females with their antennae removed are still able to react to odors, although more slowly than do normal individuals. He searched for chemoreceptors on the maxillary palps but found only what he believed to be tactile hairs. One of those shown in Fig. 32 of his paper has a rounded tip and is clearly a thick-walled peg of the kind described here.

II. Thin-walled chemoreceptors

A. Thin-walled pegs These, like the thick-walled pegs, have been found on the antennal flagellum of every insect where a thorough search has been made for them. They are transparent, or nearly so, and have a wall that approaches 0.2 μ in thickness and is penetrated by many small openings. Usually they are innervated by a group of neurons although some have been reported with only a single neuron (Boeckh, Kaissling and Schneider, 1960; Dethier, Larsen and Adams, 1963). Each dendrite branches within the peg lumen and delicate pore filaments extend, usually in clusters, from them to the pores in the wall. Here the distal tips of the filaments are exposed to the air.

Thin-walled pegs are present on all subsegments from three to eleven in both males and females. None occur on the first two or on the twelfth. They are about 25 μ long and from 2 to 2.5 μ wide at the base (Figs. 2d, 3c). Each peg is curved and lies close to the antennal wall so that its long axis nearly parallels that of the antenna itself. Jacobi (1939, Figs. 25, 26, 27) gives cross and longitudinal sections through them. Except for a short region at the base, the peg wall is perforated by many small openings. These pores are large enough to be seen easily in sections stained with silver or in whole mounts examined in glycerol (Fig. 4b).

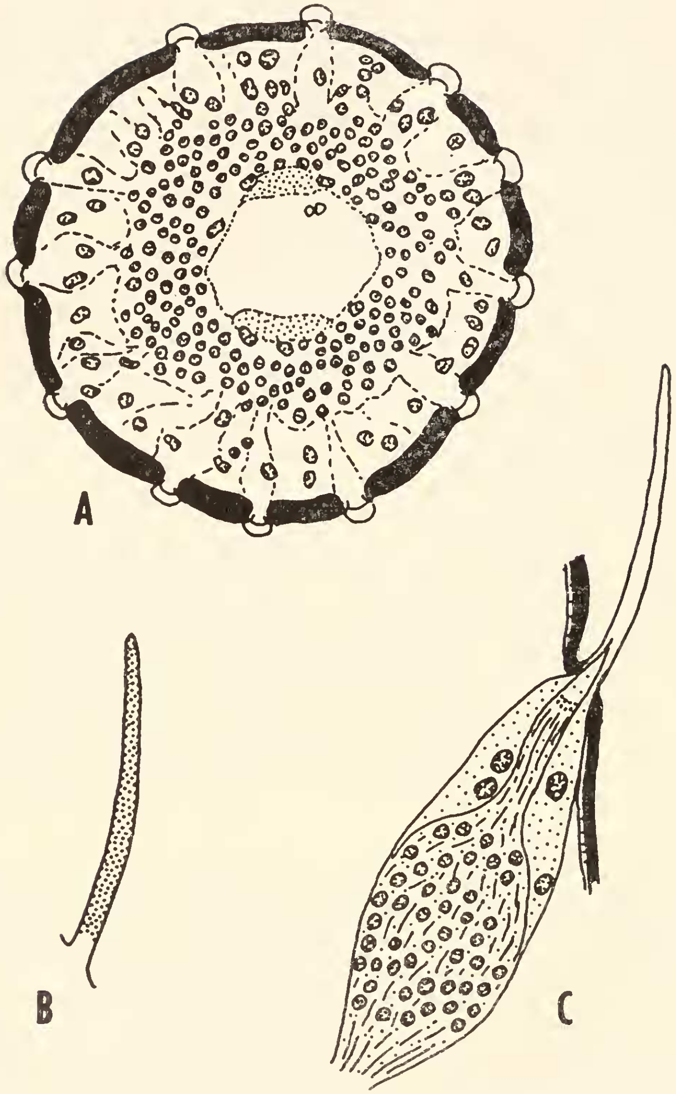

FIGURE 4. A, cross section of flagellum of female as reconstructed from several sections. Greater part of section occupied by a ring-shaped mass of sensory neuron cell bodies with small nuclei. Large nuclei are those of sheath cells. Central lumen contains two branches of antennal nerve embedded in mass of neurons. Two small circles represent tracheae. Antennal cuticle at periphery includes sections of twelve plate organs. Bouin's fixative, Holmes' silver stain. × 1163. B, whole mount of thin-walled peg from antenna of female examined in glycerol. Pores in wall appear as dark spots on a pale background. Note absence of pores at base. 5% formalin, no stain. × 1778. C, longitudinal section through a thin-walled chemoreceptor from antenna of male. Dendrites from mass of neurons (small nuclei) can be traced to base of peg. Basal bodies (small granules) present on dendrites. Larger nuclei are those of sheath cells. Bouin's fixative, Holmes' silver stain. × 1778.

Stain applied to the outside surface enters the peg readily through them. The thin-walled peg is innervated by a large group of neurons that may lie directly below it (Fig. 4c) or be merged with the larger mass of neurons from many receptors that occupies much of the lumen of the antenna. Several sheath cells (trichogen or tormogen) enclose the dendrites as they approach the base of the peg. Jacobi (1939) shows a group of basal bodies—formerly known as *Riechstäbchen, Sinnesstäbchen* or sense rods—on the dendrites below the peg base and above a large group of neurons. Basal bodies were also seen in the present study in this position (Fig. 4c). We now know, from electron micrographs of antennae of other species of insects, that the sensory dendrite narrows and assumes a ciliary structure immediately above the basal bodies.

B. Plate organs Plate organs in the Hymenoptera have attracted the attention of many workers with the light microscope during the past century but few studies of their fine structure have been made since the electron microscope has been in use. Slifer and Sekhon (1960, 1961) examined the plate organs of the honey bee, *Apis mellifera,* with the electron microscope but staining methods for sections were not available at the time and the work should be repeated. References to earlier literature on the plate organs of various species of ants, wasps and bees may be found in the paper published in 1961.

The plate organ of the honey bee consists of a thick, transparent, oval plate attached to the adjacent cuticle by a thin narrow membrane with radiating lines of minute pores in it. Beneath this thin membrane, but not beneath the plate itself, lies a mass of slender dendrite branches. These arise from a group of sensory neurons that lie below the plate. Recent physiological studies indicate that the plate organ of the honey bee serves as an olfactory organ (Lacher, 1964; Lacher and Schneider, 1963). If this is true, it is highly probable that delicate pore filaments extend from the dendrites to the pores as they do in the plate organ of the aphid (Slifer, Sekhon and Lees, 1964). The function of the thick, transparent plate that makes up the greater part of the surface of the receptor in the honey bee remains unknown.

In *Nasonia* plate organs are present on all of the subsegments except the first, second and twelfth. Jacobi (1939) counted the plate organs on subsegments three to eleven for a single antenna of a female. The results, in order, were 6, 5, 6, 6, 9, 9, 14, 14 and 5 with a total of 74. In the present study the plates on six antennae from males and six from females were counted. The results are shown in Table I. It will be noted that, with a few exceptions, the number of receptors rises for successive subsegments and then falls sharply at the eleventh. The mean total for males is 43 and for females 81. According to Cousin (1933), the removal of the antennae from a female suppresses reflexes that make mating possible but this is not the case for the male. It would be interesting to know whether the difference in the number of plate organs is in any way concerned. Perhaps they aid the female in finding decomposing material in which dipteran pupae may be found although it should be noted that Cousin (1933) states that females without antennae are still able to find such pupae.

The plate organs of *Nasonia* are the largest and most conspicuous of the antennal receptors (Figs. 2e, 3d). They are transparent, elongated structures that are raised above the antennal surface and arranged, more or less regularly, around the subsegments (Figs. 3, 4a). In life they stand out conspicuously against the dark antennal cuticle. The mean length of those measured on an antenna from a female was 31 μ and the same figure was obtained for the antenna of a male. Their maximum width is about 5 μ. When the insect is immersed in a solution of crystal violet, the stain enters rapidly through the plate organs. This provides evidence that the plate has pores in it and that the structures serve as chemoreceptors. In sections cut in a plane parallel to the surface of the antenna and stained with Holmes' silver technique the porous nature of the plate is definitely confirmed (Fig. 5a). The pores are smaller than are those of the thin-walled pegs and careful focusing of the microscope is necessary before they can be seen clearly. The entire outer surface is uniformly covered with the small pores. The relative ease

TABLE I

Number of plate organs on flagellar subsegments of antennae of males and females

Subsegment	3	4	5	6	7	8	9	10	11	Total
♂♂	3	2	4	5	5	4	6	5	2	36
	5	4	5	6	4	4	6	7	3	44
	4	4	4	5	5	7	6	6	3	44
	3	3	5	5	6	6	7	6	3	44
	2	3	3	4	7	5	7	6	3	40
	5	4	4	6	7	8	7	7	4	52
									Mean total	43
♀♀	4	5	5	8	9	11	10	13	4	69
	3	5	5	7	11	11	11	13	3	69
	7	6	8	7	10	10	15	15	6	84
	5	5	8	9	12	13	14	13	5	84
	6	7	8	8	12	12	15	14	6	88
	5	7	7	8	12	14	15	15	6	89
									Mean total	81

with which pores can be demonstrated in *Nasonia* contrasts strongly with the situation in the honey bee plate organ where it has not yet been possible to demonstrate pores with the light microscope.

In cross sections of the antenna a thin membrane can be seen extending across the receptor about 1.5 μ below the outer surface (Figs. 4a, 5d). This lies just above two shelf-like invaginations of the cuticle that may help to support it. Near the proximal end of the plate a large group of dendrites extends upward towards the membrane (Figs. 5c, 5d). Presumably these pass through one or more openings in the inner membrane as they do in the aphid plate organ (Slifer, Sekhon and Lees, 1964), then enter the outer chamber, branch and send filaments into the fine pores in the surface.

Basal bodies are present in the dendrites and may be seen as a band of dark spots 4 or 5 μ below the surface of the receptor (Fig. 5d). Jacobi (1939) evidently did not see them for there are none in his longitudinal section of a plate organ. The dendrites of the sensory neurons of the plate organs, as well as those

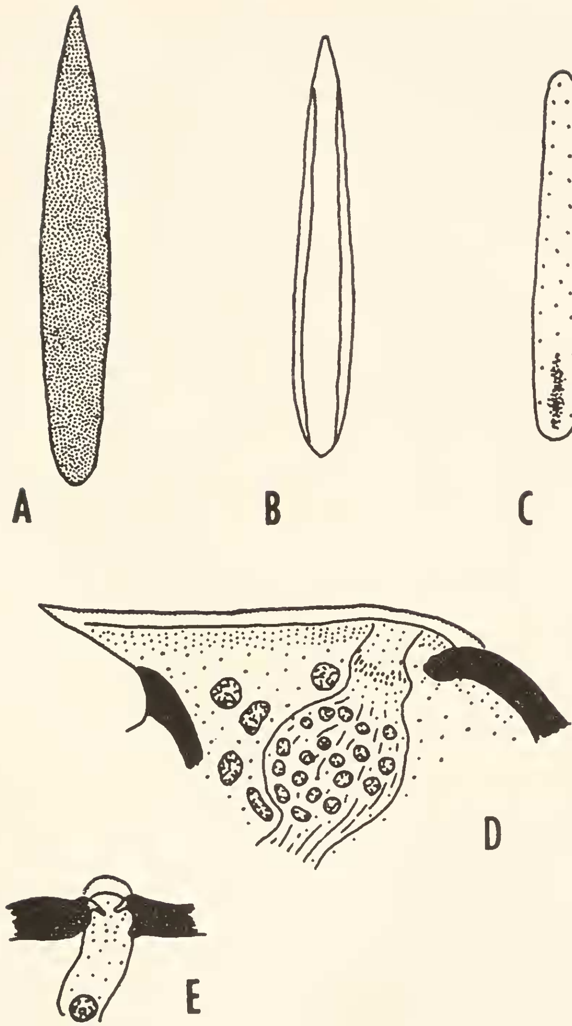

FIGURE 5. A, surface view of plate organ showing fine pores in wall. Compare with D and E. B, view through plate organ at lower focal level than that shown in A. Note two shelf-like cuticular extensions along sides. Compare with E. C, view through plate organ at still lower level and showing cluster of basal bodies at proximal end. Compare with D. D, longitudinal section of plate organ showing surface with fine pores and delicate inner membrane that separates outer chamber from parts below. The mass of neurons (small nuclei) sends dendrites toward the outer chamber. Basal bodies (small granules) present on dendrites. Larger nuclei are those of sheath cells. Compare with A, B, C and E. E, cross section through outer part of plate organ to show relations of surface with pores, delicate membrane that forms floor of outer chamber and shelf-like cuticular extensions just below it. Dendrites not included in this section. Nucleus is that of a sheath cell. All Bouin's fixative, Holmes' silver stain. × 1850.

of other receptors on the antenna of *Nasonia,* are not enclosed within a tubular cuticular sheath as they are in many insect species. This was also noted for the sense organs of *Apis mellifera* investigated earlier (Slifer and Sekhon, 1961). The group of neurons that innervates the plate organ is large and may lie directly below the plate or, more often, form a part of the compact and massive ring of neuron cell bodies that occupies much of the antennal lumen (Fig. 4a). The sheath cells that are responsible for the secretion of the cuticular parts of the plate organs encircle the dendrites and fill the region below the inner membrane. Their nuclei are larger than are those of the neurons and their free borders are covered with microvilli. The epidermal cells that lie below the antennal cuticle elsewhere are much flattened and difficult to see in sections although their nuclei can be identified.

The arrangement of the mass of neurons within the lumen of the antenna is of some interest. A single large antennal nerve traverses the scape, pedicel and first two flagellar subsegments. In the second subsegment a small mass of neuron cell bodies lies at one side of the nerve. In the third subsegment the nerve branches into two and these, decreasing in size, can be traced into the tenth subsegment. Within each subsegment from the third to the eighth the nerves are surrounded by and embedded in a large ring-shaped mass of neuron cell bodies (Fig. 4a). The masses are larger in the females since in this sex there are nearly twice as many plate organs as there are in the males. Between subsegments, where the antenna is strongly constricted, the two antennal nerves are the only sensory elements present. The ninth and tenth subsegment each contains a large mass of neurons but those of the eleventh, combined with those of the twelfth, lie in the distal end of the tenth and proximal ends of the eleventh subsegments.

Summary

1. Although the male of *Nasonia vitripennis* is distinctly smaller than the female, the antennae are approximately the same size in both sexes.

2. Hairs of two kinds, both believed to be tactile, are present on the antennae of both males and females. One type is found only on the twelfth subsegment.

3. Chemoreceptors of three kinds—thick-walled pegs, thin-walled pegs and plate organs—occur on the antennal flagellum of both sexes.

4. Thick-walled pegs are restricted to the eleventh and twelfth subsegments of the antenna. Elsewhere on the body they were found on the terminal segments of the maxillary and labial palps and on the tarsal claws.

5. Thin-walled pegs are present in large numbers on all subsegments from the third to the eleventh.

6. Plate organs occur on all subsegments from the third to the eleventh. A mean number of 43 plate organs was found on the antenna of the male and 81 on the antenna of the female. The entire outer surface of the plate is perforated by a large number of very small openings.

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